

Effects of Sublethal Exposure to Imidacloprid on Subsequent Behavior of Subterranean Termite *Reticulitermes virginicus* (Isoptera: Rhinotermitidae)

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ABSTRACT Experiments were conducted to determine whether subterranean termites, *Reticulitermes virginicus* (Banks), previously exposed to sublethal doses of imidacloprid (Premise), and allowed to recover for 1 wk, demonstrated behavioral aversion to a subsequent exposure. Worker termites experiencing a previous sublethal but debilitating exposure to imidacloprid-treated sand (either 10 or 100 ppm for 4 h) showed no apparent aversion to a second encounter with imidacloprid-treated sand under conditions of this experiment. If these laboratory results hold in the field and termites traveling through a zone of soil treated with imidacloprid are impaired but subsequently recover, they will be just as likely as their naive nestmates to reenter the treated area if their travels take them through the nonrepellent application a second time. Our results also indicate that a sublethal exposure to imidacloprid can affect termite tunneling behavior. Many worker termites that received an initial 4-h exposure to 100 ppm imidacloprid-treated sand died, but those that survived tunneled significantly less than did their naive nestmates, as did some termites exposed to 10 ppm imidacloprid.

KEY WORDS *Reticulitermes virginicus*, repellency, aversion, learning, imidacloprid, Premise

EXPOSURE TO a pesticide can result in repellency or aversion of the pest to that compound. Repellency is a product of the chemical attributes of the substance and it may occur the first time that the chemical is encountered. Aversion is a learned response after an experiential association between one or more of the compound's attributes and a negative impact such as sickness. Pesticide repellency and aversion are important because if contact between the pest and the compound is required for treatment to be effective, as is the case in termite control with nonrepellent termiticides such as imidacloprid (Premise), behaviors that keep the insects away from the pesticide may compromise efficacy.

The objective of this research was to determine whether subterranean termites, *Reticulitermes virginicus* (Banks), previously exposed to sublethal doses of imidacloprid demonstrated behavioral aversion to a subsequent exposure. We designed this experiment with the assumption that imidacloprid is not repellent to subterranean termites at the concentrations presently used to treat structures (Kard 1998, Kuriachan and Gold 1998, Potter 1999). In this study we tested whether individual termites that had prior experience with imidacloprid were deterred from tunneling or traveling in imidacloprid-treated soil. Our research question was whether termites that have suffered the effects of a previous, sublethal exposure to imidacloprid show behavioral aversion to subsequent exposures.

Materials and Methods

Termite Colonies and Individual Paint Markings. *R. virginicus* workers were collected from three field colonies within 4 mo of the experiment. PVC cylinders containing rolled corrugated cardboard were used to accumulate and collect termites from each colony. Colony I was from Whitehall, Clarke County, GA; colony II was collected in Oconee National Forest, Oconee County, GA; and colony III was located on the University of Maryland campus, College Park, Prince Georges' County, MD. Wet and dry weights of three samples of 10 workers and 10 soldiers were determined for colonies II and III and for workers only of colony I. Average worker weights (wet weight, dry weight) were as follows: colony I, 0.0022 g, 0.0005 g; colony II, 0.0021 g, 0.0005 g; and colony III, 0.0024 g, 0.0006 g. Average soldier weights (wet weight, dry weight) were 0.0026, 0.0006 for colony II, and 0.0029, 0.0008 for colony III. Termites to be exposed to Premise treated sand, or to be used in the control exposure (0 ppm Premise treated sand), were individually marked with a dot of Testor's model paint (The Testor Corporation, Rockford, IL) on their abdomen.

Initial Exposure to Imidacloprid-Treated Sand. Three clear plastic "exposure" containers (15.5 cm in radius, 6.5 cm in height) containing loosely packed sand and wood were set up for each colony. Sand (93% sand, 2% silt, and 5% clay; Maryland Cooperative Extension Service Soil Testing Laboratory) was field

collected from a deposit in park land located northeast of the termination of Plateau Place, Greenbelt, MD, within 3 d of the experiment. Exposure containers received 200 g of air-dried sand mixed with 20 ml of distilled water or 20 ml of the appropriate imidacloprid solution (Premise; Bayer, Kansas City, MO) to yield 0, 10, or 100 ppm imidacloprid treatments. Mixing was done by manipulating sand and liquid in sealed 1-g plastic food storage bags. The maximum label rate for initial soil application of Premise is 100 ppm. Twenty grams (dry weight) of decayed white birch (*Betula papyrifera* Marsh.) was moistened and added to the sand as food in each container. The sand and wood covered the base of the container to a depth of ≈ 1.5 cm. Four hundred individually painted termites were placed in each of the three containers (0, 10, and 100 ppm imidacloprid-treated sand + birch wood), and the container was covered with a tightly fitting lid.

Transfer to "Recovery" Containers with Naïve Nestmates. After the 4-h exposure to control (0 ppm) or imidacloprid-treated sand (10 and 100 ppm), 300 termites (all still marked with paint) were removed from the exposure container with forceps and placed in "recovery" containers (size identical to exposure containers). The recovery container had 200 g of wild, air-dried, untreated sand (collected at the same time and source as the treated sand) mixed with 20 ml of distilled water and 20 g (dry weight) of moistened, decayed white birch. To prevent use as a refuge the birch was chopped into fragments < 2 mm in any dimension and incorporated into the sand when the liquid was added. Each recovery container also housed 600 naive (no previous exposure to imidacloprid), healthy, unpainted nestmate worker termites.

The painted, imidacloprid-exposed and control worker termites and their unpainted, healthy nestmates were kept in the recovery container for 1 wk after removal of the painted termites from the exposure container. Each of the three recovery containers for each of the three colonies was monitored at 24-h intervals, at which time dead termites (if any) that were on the surface of the sand were counted and removed.

Behavioral Response to Imidacloprid. Imidacloprid causes blockage of insect-specific nicotinic-acetylcholine receptors, with resulting tetanic muscle contractions within minutes of exposure. Lethal doses cause intense trembling of the legs and pumping movements of the body with attendant nerve and muscle destruction due to the hyperactivity (Mehlhorn and Mencke 1999). Sublethal doses due to concentration and length of exposure affect mobility with resulting behavioral changes such as reduced or impaired grooming, feeding, tunneling, and walking. As exposure times and/or concentrations increase termites become immobile, and lay on their backs with occasional twitching of legs and antennae. Death may take several days depending on humidity levels and presence of invasive organisms like nematodes and fungi. In all cases termite mobility was described in comparison to the control treatment(s).

Behavioral Bioassay to Examine the Effect of Previous Exposure to Imidacloprid. After 7 d in a recovery container with naive nestmates, active, marked termites (paint marks differentiating workers previously exposed to 0, 10, or 100 ppm imidacloprid from the naive nestmates in the recovery arena) were removed to participate in a bioassay designed to assess the effect of previous exposure to imidacloprid. Each bioassay arena consisted of an 8.5-cm-diameter plastic petri dish bottom nested within an 8.9-cm-diameter clear, round plastic container lid. This arrangement created a space ≈ 3 mm in depth between the lid surfaces to fill with sand for the tunneling bioassay. One half of this space was packed with wild, untreated sand; the other half was filled with wild imidacloprid-treated sand. All sand was field collected from the same location in Greenbelt, MD, within 3 d of the bioassay, air-dried, and moistened using a proportion of 200 g of dry sand + 20 ml of liquid (imidacloprid solution for treated sand, distilled water for untreated sand). The sand halves (treated or untreated) abutted each other seamlessly, and were evenly packed, allowing the top plate to tightly contact the sand surface. No wood was added to the bioassay plates. In the center of the sand-filled plate a circular void (1.0 cm in diameter) was kept open as a space to release termites into the arena.

For each colony and each previous exposure concentration (0, 10, or 100 ppm), we ran five replicate bioassays by using four concentrations of imidacloprid (0.0, 0.1, 1.0, and 10.0 ppm) in the treated-sand half of the bioassay arena. (An exception was that all of the colony II termites in the 100 ppm exposure treatment and their nestmates in the recovery arena died, hence that treatment was omitted from the tunneling bioassay; see Results.) Ten termites were introduced into the void in the center of the sand in each replicate, and the petri plate was immediately replaced.

The number of tunnels initiated (starts), tunnel distance (millimeters), and location of tunneling (in treated or untreated sand) in each plate was recorded at 1, 5, 18, and 24 h after introduction of termites into the bioassay arena. Tunnel locations were traced directly on the plate using a different color marker for each monitoring time, and tunnel starts, distances, and locations were subsequently evaluated. Measurements on individual tunnels stopped after the tunnel reached the outer edge of the arena because it was observed that subsequent tunneling simply followed the perimeter of the arena with little or no additional interior tunneling.

Herein, we report on progress at 5 h (inclusive, i.e., sum of the tunneling at 1 h plus that occurring in the 1- to 5-h interval). After only 1 h the termites appeared to still be acclimating, and by 5 h the effect of the imidacloprid was noticeably reduced tunneling at the higher concentrations on the treated halves of the bioassay arenas, leading us to question the relevance of the 18- and 24-h data. Mean percentage of tunneling distance and starts were calculated as the fraction of tunnel length (or number of starts) in the untreated

Table 1. Percentage of painted, imidacloprid exposed workers surviving 1 wk after entering recovery arena (containing 300 painted, exposed and 600 unpainted, naive termites)

Colony	Pre-exposure levels, ppm								
	0			10			100		
	Painted ^a	Unpainted ^b	Total	Painted ^a	Unpainted ^b	Total	Painted ^a	Unpainted ^b	Total
I	92.0	100	98.1	67.0	93.2	95.6	68.3	95.3	86.3
II	82.7	93.7	90.0	82.3	90.5	87.8	—	—	—
III	90.7	99.8	96.8	80.3	94.7	89.9	82.0	95	91.8

—, All colony II termites died with the initial pre-exposure of 100 ppm.

^aConservative estimate because some of the termites lost their mark due to grooming or molting and were included in the unpainted category.

^bMay include painted termites that lost their mark.

side of the bioassay divided by total tunneling in both sides of the bioassay arena.

Statistical Methods: Survival of Termites After 1 wk in Recovery. A PROC Mixed procedure (version 6.12, SAS Institute 1988) was used to run an analysis of variance (ANOVA) to compare the percentage of termites surviving at the end of 1 wk in the recovery arena (after initial exposure to imidacloprid-treated sand, or the control treatment in wild sand).

Tests for Repellency and Aversion. To test whether a preference existed for starting tunnels and/or tunneling in the untreated sand of the bioassay arenas by termites following 1 wk in "recovery" a PROC Mixed procedure was used to run an ANOVA. Within this analysis colonies were treated as the highest order of replication, and termites from all three colonies that survived the initial exposure to imidacloprid responded similarly within bioassay treatments. Schwarz's Bayesian criterion was the goodness-of-fit test used for deciding whether to partition the variances based on differences between parameters (initial exposure and concentration of imidacloprid in the treated half of the bioassay plate), or to pool variances as a single residual variance. The PROC Univariate was run to check for normality.

Total Distance Tunneled and Total Number of Starts. The data were analyzed using the PROC ANOVA procedure. The independent variables were colony (I, II, and III), initial exposure concentration (0, 10, and 100 ppm), and concentration of imidacloprid on the treated half of the sand in the bioassay arena (0.0, 0.1, 1.0, and 10.0 ppm). Data on the mean total length of tunnels in treated and in untreated sand and the mean number of tunnel starts for the five replicates of each treatment were analyzed with ANOVA, followed by a Duncan multiple range test to consider differences among concentrations of Premise in the bioassay arenas.

Results

Behavior of Termites After 4 h of Exposure to Imidacloprid-Treated Sand. After 4 h of exposure to imidacloprid-treated sand, mobility of the termites depended on both concentration and colony of origin. Control treatment (0 ppm) termites tunneled below the surface within 4 h. All termites exposed for 4 h to 10 ppm imidacloprid-treated sand displayed symp-

toms. Every termite in colonies I and II was immobile (unable to walk) or had impaired mobility (slow, staggering gait) compared with the controls. In colony II the exposed termites all had impaired mobility compared with the controls but none were immobile. After 24 h in the recovery arena, the mobility of 95% of exposed and naive termites from all colonies was indistinguishable from controls.

After 4 h of exposure to 100 ppm imidacloprid-treated sand, termites from two of the colonies displayed severe symptoms. All workers from colonies I and III were immobile on the surface of the sand. At the end of 4 h, termites from colony II were still mobile (impaired) in the 100 ppm imidacloprid-treated sand.

After 24 h in all three colonies, 95% of the individuals in the 100 ppm recovery arena (both painted and unpainted termites) remained on the surface with the painted (exposed) workers. Of these exposed workers 96% were immobile and 4% showed impaired mobility. More than 40% of the unpainted naive nestmates were impaired as evidenced by slow, staggering gait and their presence on the surface instead of in tunnels. These observations of impairment in naive workers provide further evidence that termites that are indirectly exposed to imidacloprid through contact with exposed nestmates also may have their health and behavior affected by imidacloprid. Onset of symptoms and, in some cases, death among naive termites in the recovery arenas suggests that residues of imidacloprid are being transferred between termites, although the specific mechanism(s) of transfer cannot be elucidated from our experiment.

In colonies I and III, 95% of the termites stayed on the sand surface throughout the week in the 100 ppm recovery container, with 86.3% surviving in colony I and 91.8% in colony III, and all individuals dying in colony II. In the same treatment for colony II, 98% of the workers stayed on the sand surface for 48 h after entering the recovery arena, with 100% back on the surface at 72 h, but all but 20 of the live termites entering the sand by 4 d. The total number of dead on the sand surface removed by the end of 7 d in recovery were 32 termites in colony I and 27 termites in colony III; all died in colony II.

Survival of Termites in Recovery Groups After 1 wk. Table 1 shows survivorship of workers alive 7 d after entering the recovery arena (initially containing 300 painted workers exposed to 0, 10, or 100 ppm

imidacloprid-treated sand and 600 naïve nestmates). A significantly higher percentage of unpainted versus painted termites was present after 7 d in the recovery arena in all treatments including the control ($P < 0.0055$). However, the number of exposed survivors, as measured by counts of painted workers, is conservative. Some individuals may have lost their paint marks due to abrasion or grooming, and therefore are inadvertently included with the unpainted group. This definitely occurred in the colony I control treatment (0 ppm imidacloprid), in which more unpainted termites (607) were retrieved than were originally placed in the recovery container (600). Thus, the number and percentage of exposed workers are minimum estimates of survivorship, and the data for unpainted workers may be exaggerated due to inclusion of termites that lost their mark. There was no significant interaction between any level of initial exposure to imidacloprid and whether the termites were marked with paint.

The overall count and percentage of survivorship of the 900 termites originally placed in the recovery arena are precise. Overall survivorship of termites from all colonies and in all treatments was high, exceeding 82% (exposed + naïve) in all cases, except for colony II, in which all termites in the 100 ppm recovery arena died within 4 d. As expected, survivorship in the control treatment for all colonies exceeded that in groups containing termites exposed to imidacloprid-treated sand. In all cases, both painted and unpainted termites alive in the recovery container at the end of 1 wk appeared healthy and active.

Tunneling Bioassays: Repellency. If termites are repelled by imidacloprid, two possible results are expected. Naïve (control) termites would show a consistently higher percentage of tunneling or a higher percentage of starts in the untreated sand versus treated sand of the bioassay plates. Alternatively, or in addition, there may be a concentration-dependent response, with an increasing percentage of distance tunneled and/or number of tunnel starts in the untreated sand with increasing concentration of imidacloprid on the treated side.

Our results (Fig. 1 A and B) show that the control termites (painted and initially exposed to 0 ppm imidacloprid, i.e., untreated sand) behave with no bias in either distance tunneled or number of tunnel starts in bioassay plates with 0.1 ppm imidacloprid on the treated half of the plate. In the bioassay plates with 1.0 ppm imidacloprid on the treated half, there was a bias toward both tunneling distance (expressed as mean percentage of tunneling) and percentage of tunnel starts in the untreated side of the arena ($P < 0.01$ for distance; $P < 0.05$ for starts). There was a bias toward greater tunneling distance and starts in the treated sand in bioassays containing 10.0 ppm imidacloprid ($P < 0.01$ for distance; $P < 0.001$ for starts).

Aversion. If aversion or a learned response to imidacloprid is occurring, one expects a higher percentage of tunneling or a higher percentage of tunnel starts in the untreated sand by termites previously exposed to imidacloprid than by their naïve (control) nest-

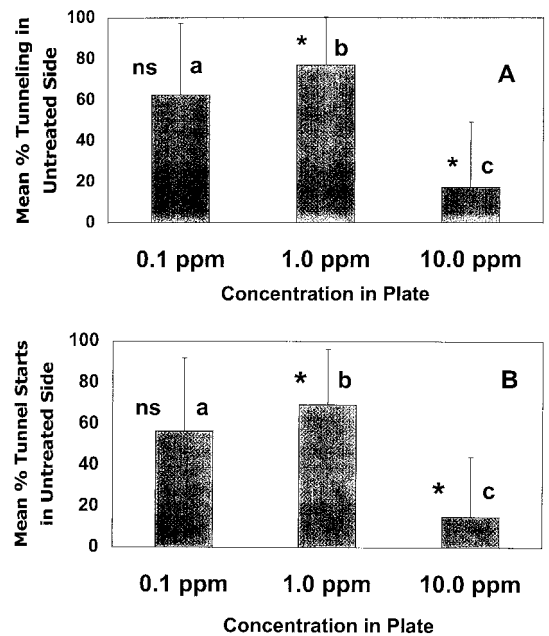


Fig. 1. Mean percentages (\pm SD) of (A) tunneling and (B) tunnel starts in the untreated side of the bioassays for each concentration by naïve termites. Significant differences from unbiased tunneling (50%) within bioassay concentrations are indicated by ns or *. Comparisons among concentrations are labeled with letters. Concentrations with the same letter are not significantly different, Duncan multiple range test, $P = 0.05$ (SAS Institute 1996).

mates. As with repellency, the results regarding aversion to imidacloprid-treated sand are consistent for both distance tunneled (Fig. 2 A and B) and number of tunnel starts (Fig. 3 A and B) in the bioassays. Termites initially exposed to either 10 or 100 ppm imidacloprid-treated sand and then allowed to recover with naïve nestmates for 1 wk showed no significant bias to either 0 ppm or 0.1 ppm imidacloprid in the treated side of the bioassay plate. There was also no significant bias in these measures of preference for untreated versus treated sand by termites initially exposed to 100 ppm imidacloprid and then tested in bioassay plates with 1.0 ppm imidacloprid in the treated half. Termites initially exposed to 10 ppm imidacloprid-treated sand showed a bias toward both tunneling distance ($P < 0.01$) and percentage of tunnel starts ($P < 0.05$) in the untreated side of the bioassay plate when the concentration of imidacloprid in the treated half was 1.0 ppm. Termites initially exposed to either 10 or 100 ppm imidacloprid showed a preference for the treated side of the bioassay plates containing 10 ppm imidacloprid (10 ppm: $P < 0.05$ for distance, $P < 0.01$ for percentage of starts; 100 ppm: $P < 0.0005$ for distance, $P < 0.001$ for starts). Thus, there is no suggestion of avoidance behavior associated with prior exposure to imidacloprid.

Distance Tunneled. Total distance tunneled (on the treated + untreated sides of the bioassay areas) was evaluated for all colonies after 5 h. For colony I, ter-

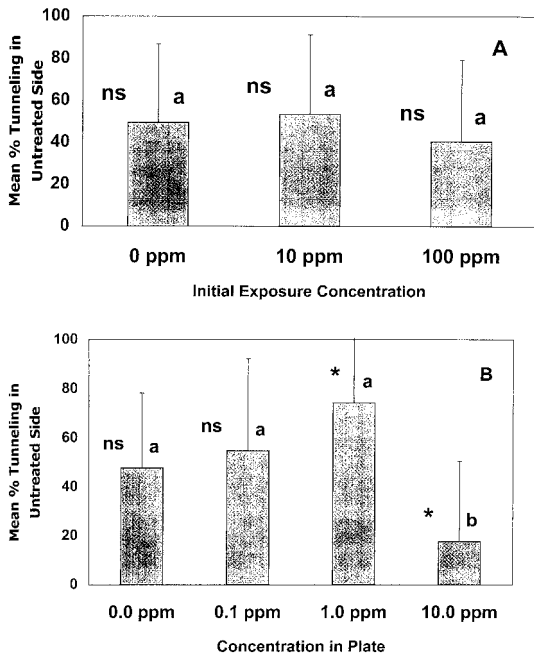


Fig. 2. Mean percentages (\pm SD) of tunneling in the untreated side of all bioassay replicates based on (A) initial exposure concentration (sum of tunneling in all bioassay plates) and (B) concentration in the treated side of the bioassay. Significant differences from unbiased tunneling (50%) within bioassay concentrations are indicated by ns or *. Comparisons among concentrations are labeled with letters. Concentrations with the same letter are not significantly different, $P = 0.05$ (SAS Institute 1996).

mites previously exposed to 100 ppm imidacloprid-treated sand for 4 h, and then allowed to recover with naive nestmates for 1 wk, tunneled significantly less than nestmates initially exposed to either 0 or 10 ppm imidacloprid ($F = 4.85$; $df = 11, 48$; $P = 0.0001$). In colony II, the colony in which all termites exposed to 100 ppm imidacloprid-treated sand died before the end of their week in recovery, there was similarly no significant difference in amount of tunneling between those workers initially exposed to 0 or 10 ppm imidacloprid-treated sand. For colony III, results also showed a significant effect of initial exposure concentration, with control termites (0 ppm exposure) tunneling significantly more than either the 10 ppm or 100 ppm exposed workers (Fig. 4A). Thus, even after 1 wk of "recovery time" the termites exposed to 100 ppm imidacloprid, and sometimes even at 10 ppm, still display sublethal effects on tunneling behavior.

For colony I-III, a Duncan multiple range test showed that there was significantly less tunneling in bioassay plates with the highest imidacloprid concentration (10 ppm) than in any of the treatments with a lower concentration of imidacloprid ($P = 0.05$) (Fig. 4B). In no case was the interaction effect (of initial exposure concentration) significant, thus the concentration in the bioassay plates affected exposed and naive termites in the same way. On a practical level,

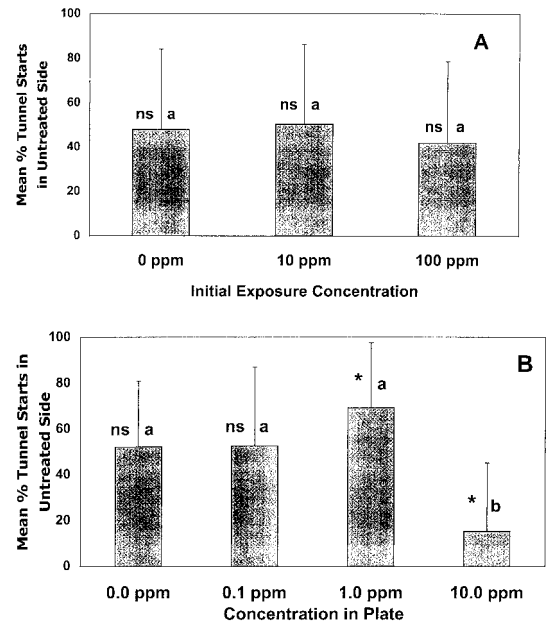


Fig. 3. Mean percentages (\pm SD) of tunnel starts in the untreated side of all bioassay replicates based on (A) initial exposure concentration (sum of tunneling in all bioassay plates) and (B) concentration in the treated side of the bioassay. Significant differences from unbiased tunneling (50%) within bioassay concentrations are indicated by ns or *. Comparisons among concentrations are labeled with letters. Concentrations with the same letter are not significantly different, $P = 0.05$ (SAS Institute 1996).

this result demonstrates that exposure to just 10 ppm imidacloprid can suppress tunneling activity within 5 h.

Number of Tunnel Starts. The number of tunnels initiated or started (sum of treated + untreated sides of the bioassay arena) was counted after 5 h. For all colonies, the number of tunnel starts did not significantly vary among termites differing in concentrations of initial exposure to imidacloprid (0, 10, or 100 ppm) (Fig. 5A). In all colonies, termites in bioassays with 10 ppm imidacloprid-treated sand had significantly fewer tunnel starts than in bioassay plates with lower concentrations of imidacloprid-treated sand (Fig. 5B). Again, on a practical level, this result shows that exposure to just 10 ppm imidacloprid can inhibit tunnel initiation within 5 h.

Discussion

Reticulitermes virginicus workers exposed to 10 ppm imidacloprid-treated sand for 4 h have a high chance of surviving treatment if the exposure is terminated and they are subsequently allowed to recover for 1 wk in an arena of sand, wood, and a large number of naive nestmates. For two of the three colonies we studied, this ability to recover was also true for termites exposed to 100 ppm imidacloprid-treated sand for 4 h. This recovery may parallel the field situation in which a portion of a colony travels though imidacloprid-

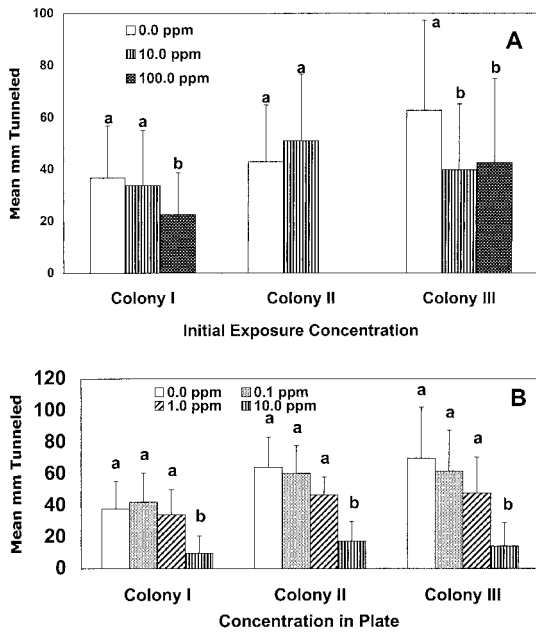


Fig. 4. Mean mm tunneled (\pm SD) (sum of tunnel length in treated + untreated side of bioassay plate) based on (A) initial exposure concentration and (B) concentration in the bioassay. Within colonies, concentrations with the same letter are not significantly different, $P = 0.05$ (SAS Institute 1996).

treated soil, obtaining a sublethal dose of the pesticide before returning to their colony to interact with healthy nestmates and potentially transfer imidacloprid to naive nestmates. It differs from the field situation, however, in that our laboratory experiments allowed us to remove live but immobilized termites from the imidacloprid-treated sand and place them in untreated sand with healthy, naive nestmates. If immobilized by imidacloprid in the field, termites may not be able to escape the treated soil to mingle and perhaps recover with naive nestmates. Our experiments are thus most relevant to the field situation in which termites make a relatively rapid (≤ 4 h) pass through a segment of imidacloprid-treated soil and are not immediately immobilized but are still affected.

Colonies differ in the susceptibility of their workers to exposure to 10 and 100 ppm imidacloprid-treated sand. This result is not simply a function of average size of termites in a colony; in our study individuals of colony I and II were of identical average size (wet weight and dry weight), yet all 100 ppm exposed workers died in colony II. The continual activity of colony II termites compared with the immobile colonies I and III may have exposed them to more imidacloprid in 4 h.

Worker termites experiencing a previous sublethal but initially debilitating exposure to imidacloprid-treated sand (either 10 or 100 ppm for 4 h) show no apparent aversion to a second encounter with imidacloprid-treated sand under conditions of this experiment. The response of previously exposed termites

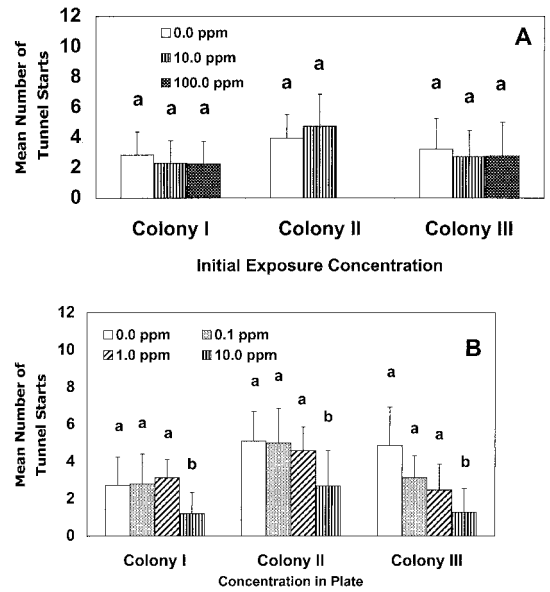


Fig. 5. Mean number tunnel starts (\pm SD) (sum of tunnel starts in treated + untreated side of bioassay plate) based on (A) initial exposure concentration and (B) concentration in the bioassay. Within colonies, concentrations with the same letter are not significantly different, $P = 0.05$ (SAS Institute 1996).

closely parallels the response of naive termites to differing concentrations of imidacloprid-treated sand, suggesting that there is no "learning" of an avoidance response to this insecticide, at least within the exposure levels and circumstances of this laboratory study. Notably, both naive and experienced termites presented with a choice of untreated or 10 ppm imidacloprid-treated sand (the highest concentration used in our bioassay) significantly preferred tunneling in the treated sand. If our laboratory results hold in the field, they suggest that if termites travel through soil treated with imidacloprid and are impaired but subsequently recover, they will be just as likely as their naive nestmates to reenter the treated soil if their travels take them through the imidacloprid application a second time.

Recovered workers that had received an initial 4-h exposure to 100 ppm imidacloprid-treated sand tunneled significantly shorter mean distances than did their naive nestmates (demonstrated in colonies I and III; colony II termites exposed to 100 ppm imidacloprid-treated sand all died in recovery). In colony III, even a 4-h exposure to 10 ppm imidacloprid-treated sand followed by a 1-wk recovery resulted in reduced tunneling distance. These data suggest that sublethal contact with imidacloprid can affect at least two behaviors among termites surviving the exposure: reduced grooming and removal of fungal spores (Boucias et al. 1996) and reduced tunneling. Accumulation of fungal spores explains the increased susceptibility of imidacloprid-exposed subterranean termites to entomopathogenic fungi (Almeida and Alves 1996, Boucias et al. 1996, Almeida et al. 1998, Ramakrishnan et

al. 1999). Reduced tunneling after sublethal imidacloprid exposure would impact subterranean termite productivity, and reflects another residual effect of a compromised neurological system resulting from imidacloprid exposure (Schroeder and Flattum 1984).

For all colonies, termites tunneled shorter distances and initiated fewer starts in bioassay plates containing 10 ppm imidacloprid-treated sand than in bioassays with a lower concentration of imidacloprid. This is almost certainly because termites exposed to 10 ppm are compromised and start to display sublethal effects well before the end of the 5-h monitoring interval.

Slow-acting, nonrepellent termiticides are a relatively recent innovation in subterranean termite control. As with fast-acting, nonrepellent termiticides such as chloropyrifos, they are designed to kill rather than merely repel termites (Su et al. 1982). Termites present in a structure when a repellent termiticide is applied are "trapped," but remain a functional, isolated fragment of the colony if they have sufficient access to moisture. In principle, this risk of a residual infestation persisting within the structure is reduced with a nonrepellent application because termites within the structure would presumably maintain their soil connection (assuming it was not a wholly aboveground infestation). Over time, workers in the structure might leave and travel through soil treated with nonrepellent termiticide, ultimately resulting in suppression of the colony and elimination of the infestation (Su et al. 1982, Potter 1999). Our data give no indication of repellency of naive termites to 0.1 ppm imidacloprid-treated sand, a bias toward the untreated side at 1.0 ppm imidacloprid, and a significant preference for 10 ppm imidacloprid-treated sand over wild sand.

A key question concerning nonrepellent termiticides has been the response of termites to sublethal doses and the effect of these termiticides on termites that travel quickly through established tunnels within the treated soil. This study suggests that, at least for imidacloprid, termites that receive a previous sublethal dose (10 or 100 ppm exposure for 4 h) will not be averted if they reencounter an imidacloprid-treated zone. The capacity of the previously exposed termites to build new tunnels is compromised if they receive a high enough initial exposure to imidacloprid. Further studies are necessary to examine effects of exposure times and concentrations beyond those included in this study, and to confirm that these laboratory results accurately reflect behavioral responses under field conditions.

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